Docket No.: 397272000500
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Boro DROPULIC

Application No.: 10/627,940 Confirmation No.: 3674

Filed: July 25, 2003 Art Unit: 1636

For: HIGH-THROUGHPUT METHODS FOR IDENTIFYING GENE FUNCTION USING LENTIVIRAL VECTORS (AS AMENDED) Examiner: N. S. Vogel

DECLARATION UNDER 37 C.F.R. § 1.132

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

1. I, Laurent Michel Humeau, Ph.D., am an expert in the field of molecular biology and in particular in constructing and using lentiviral vectors for gene therapy, and was at the time of the invention. I am presently employed as Vice President of Research and Development at VIRxSYS Corporation, assignee of the above-referenced patent application. My resume is attached as documentation of my credentials (Exhibit A).

2. This Declaration is being filed in response to the Examiner's concerns regarding enablement that were brought up during an interview with Applicants' representative on July 17, 2007. During the interview, the Examiner expressed concern as to whether the skilled artisan at the time of the invention would know how to make a pseudotyped lentiviral vector that is "designed to express little or no vector borne sequence" other than a sequence of interest, as disclosed in the pending claims of the above-referenced application.

3. It was suggested by the Examiner that Applicants submit a § 1.132 Expert Declaration stating that with the teaching of this invention's disclosure one of skill in the art would have been able to practice the claimed invention at the time of the invention without undue experimentation.

4. It should be noted that the novelty of the invention is based, *inter alia*, on the novelty of the methods as claimed in the above-referenced application, and not on the novelty of the lentiviral vector used to practice the claimed method, and that *any* lentiviral vector, including any pseudotyped lentiviral vector, can be used to practice any of the claimed methods.

5. I declare that the skilled artisan, at the time of the invention, using the teachings of the specification and the knowledge known to the skilled artisan, would be able to make a pseudotyped lentiviral vector designed to express little or no vector borne sequence other than a sequence of interest using experiments that were routine experiments not empirical experimentation of trial and error. The routine nature of the experiments performed is described below.

6. To illustrate the state of the art at the time of the invention, Applicants herein import text expressly incorporated by reference in the specification.

7. Paragraph 20 of the specification as published (US 2004/0203017A1) refers to U.S. Patent No. 6,410,257 (hereinafter "the '257 patent"), and paragraph 85 expressly incorporates by reference all "references, including patents…."

8. The '257 patent teaches one of skill in the art how to make a vector that expresses little or no vector borne sequence other than the sequence of interest. It should be noted that the vector described in the '257 patent is only ***an example*** of *one* type of vector that can be used in the claimed methods.

9. The '257 patent discloses a vector that comprises at least one sequence to be expressed, wherein the sequence itself or the translation product of the sequence is useful in the

treatment of a human disease. This sequence is the only sequence that is desired to be expressed in a cell, because it would *not* be desirable to express anything other than the sequence of interest if the vector is going to be put into a cell of a human for the treatment of HIV (*see* column 8, lines 8-10) or cancer (*see* column 22, lines 27-47). Column 7, line 63 to column 8, line 7, states that "a nonpathogenic, conditionally replicating vector...comprises at least one nucleic acid sequence...."

10. In addition, column 11, lines 19-24, of the '257 patent discusses how a "conditionally replicating vector ... replicates only upon complementation with a wild-type strain of virus...." Furthermore, column 13, lines 3-36, describes how a conditionally replicating lentiviral vector:

> "lacks sequences encoding proteins that block superinfection with wild-type HIV (e.g., nef or env proteins) or comprises such sequence but they are either *not transcribed or not translated into functional protein*, such that their expression is deemed '*silent*.' Even more preferably, the vector *lacks the region or sequences coding the region of wild-type HIV from within the gag coding sequence to and including the nef gene*." (Emphasis added.)

> It is further described how in "[e]xample 1 ... HIV, is cleaved using restriction enzymes to excise HIV encoding sequences from within the gag coding region to within the U3 region, following the nef gene.... The resultant vector produces a truncated gag transcript, and *does not produce wild-type Gag protein, or any other wild-type HIV proteins*. Moreover, it is not necessary that the vector express even the truncated gag protein inasmuch as the *gag translation initiation sequence can be mutated to prevent its translation*." (Emphasis added.)

11. Examples of retroviral vectors useful in the treatment of HIV are provided in Figures 1B-1E of the '257 patent. In addition, Example 1, starting at column 29, line 55, to column 32, line 31, describes in detail the construction of a conditionally replicating lentiviral vector.

12. In addition to the teachings of the specification provided above, submitted herein are three articles (attached Exhibits B-D) that were published before the priority date of the current application, specifically January 25, 2001, showing that not only were many types of

lentiviral vectors known at the time, but one skilled in the art knew how to design and manipulate such a vector.

13. **Kalpana, G.V., Retroviral Vectors for liver-directed gene therapy, Semin. Liver Dis. 19(1):27-37 (1999)** (hereinafter "Kalpana").

14. In Kalpana's review article, the principles underlying the design, construction, and use of retroviral vectors for gene therapy are discussed, with an emphasis on lentiviral vectors. The article describes how retroviruses are popular gene therapy vectors and how advances in the design of retroviral vectors have resulted in them being widely used in *ex vivo* gene therapy protocols (*see* Abstract). Page 27, second column, first full paragraph, describes how a thorough understanding of retroviral replication has helped in the design of efficient retroviral vectors.

15. The Kalpana article provides multiple examples of different types of lentiviral vectors and shows how it was common for one skilled in the art, at the time of the invention, to manipulate and generate various types of lentiviral vectors.

16. Page 28, second column, first paragraph, Kalpana describes how the most effective retroviral vectors are replication-defective viral vectors that are derived by manipulating the viral genome and by replacing most or all of the viral genes by therapeutic transgenes. Such manipulation is described in the second paragraph, specifically, "the therapeutic gene of interest is cloned into the transducing vector DNA that is 'crippled' or deleted of all the viral genes necessary for replication...."

17. On Page 31, first column, first full paragraph of Kalpana, self-inactivating retroviral (SIN) vectors are described. These vectors have been "handicapped", specifically, "a deletion introduced into the U3 region to remove enhancer and promoter elements at the 3' end of the transfer vector gets transmitted to the 5' region of the viral DNA in the recipient cell during reverse-transcription. When this viral DNA integrates, it lacks both 5' and 3' U3 regions, and thus is not able to initiate transcription from the viral LTRs."

18. Page 33, second column, first paragraph of Kalpana, describes how the best studied lentivirus is HIV-1, and how a crippled version of this virus has been developed into a lentiviral vector and has been used as a vehicle for *in vivo* gene delivery.

19. The generation of lentiviral vectors is discussed further on page 34, first column, second full paragraph of Kalpana. Lentiviral vectors are described in which the transgene of interest is inserted between the LTRs and the packaging signal. In addition, the vector is pseudotyped with VSV-G to obtain a broad host cell range.

20. On page 35, first column, first full paragraph of Kalpana, second- and third-generation lentiviral vectors are described wherein many of the accessory proteins are removed from the vectors. Furthermore, if is discussed how by introducing mutations or deletions into the vector, four of the accessory proteins that are not essential for vector-mediated transduction were eliminated. The replacement of a native promoter present in the U3 region of LTR with a constitutive promoter, such as CMV, is also described.

21. On page 35, under the section entitled "Perspectives" the Kalpana describes how retroviral vectors offer great potential for gene therapy and how out of several hundred clinical gene therapy trials currently ongoing, more than 45% are using retroviral vectors. Furthermore, it is stated how "the current improvements in lentiviral vectors eliminate the possibility of regenerating RCR and thus are excellent biosafety measures." In the last paragraph of page 35, the authors note how "[a]nother flexibility of retroviral vectors is that they can be pseudotyped with envelopes from other viruses, thus expanding the possibility of host range."

22. Thus, Kalpana explains how because of the thorough understanding of retroviral replication and the structure of viral RNA, skilled artisans were able to design, manipulate, and create lentiviral vectors that are safe enough for use in gene therapy trials.

23. **Naldini, L., In vivo gene delivery by lentiviral vectors, Thromb. Haemost., 82(2):552-554 (1999)** (hereinafter "Naldini").

24. Page 552, first column of Naldini, starting at the last paragraph, describes the design and construction of a hybrid lentiviral vector comprising a core derived from HIV-1, thus maintaining the ability of the lentiviruses to infect non-dividing cells, combined with the envelope of another virus.

25. On Page 552, second column, second full paragraph, Naldini discusses how they "embarked on the identification of the minimal genetic information required for transduction. All HIV-1 sequences found unnecessary were eliminated from the constructs used to generate the vectors." The last paragraph on page 552 describes how the authors were able to successfully eliminate the nonessential sequences from the constructs used to generate the vector.

26. Thus, Naldini supports that at the time of this invention it was routine for the skilled artisan to manipulate the sequences of HIV-1 in order to design a lentiviral vector.

27. **Wong-Staal, F., *et al.*, Development of HIV vectors for anti-HIV gene therapy, Proc. Natl. Acad. Sci. USA, 93(21):11395-11399 (1996).** (hereinafter "Wong-Staal")

28. On Page 11397, second paragraph of Wong-Staal, starting at the last paragraph, the authors describe the construction of HIV-1 and HIV-2 based lentiviral vectors. Among other things, it is discussed how various manipulations to the retroviral vectors are conducted: a 5' LTR is linked to a leader sequence; a marker gene is inserted; and in some vectors an element from Mason-Pfizer monkey virus was inserted in place of an RRE. These are all examples of how one skilled in the art was able to design, manipulate and construct lentiviral vectors.

29. In summary, the skilled artisan at the time of the invention, based on the teachings of the specification and the knowledge and methods known at the time of the invention, would have known how to routinely design and make a pseudotyped lentiviral vector that is "designed to express little or no vector borne sequence" other than a sequence of interest, as disclosed in the pending claims of the above-referenced application. Lastly, one skilled in the art would not have to engage in undue experimentation to make and use the claimed invention in its full

scope – particularly with respect to making and using a lentiviral vector to practice this claimed invention.

30. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted,

Dated: October 19, 2007

Laurent Michel Humeau, Ph.D.